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Application of 4,4′-Diaminobenzanilide Schiff Base Metal Complexes As Anti-Tumor and Anti - Dengue Agents

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Abstract

A unique Schiff base ligand 4-(4-nitrobenzylideneamino)-N-(4-(4-nitroobenzy lideneamino)phenyl)benzamide L has been prepared by condensing 4,4'-diaminobenzanilide and p-nitrobenzaldehyde. The cobalt(II), nickel(II) and copper(II) complexes of L were also prepared. The formation and the structure of the ligand and its complexes were confirmed by ^{1}H-NMR, FT-IR, UV-Vis., EPR and EI- mass techniques. The synthesized complexes were docked with Human DNA topoisomerase I (PDB: 1SC7) and Dengue NS3 protease-helicase (PDB ID: 2VBC) using Auto Dock vina and Discovery studio software. The biological applications of the synthesized complexes were carried out by Cytotoxic screening analysis, DNA binding ability by using electronic spectra and Anti-Tumor activity by MTT assay. The results established that the synthesized transition metal complexes can be act as good anti-tumor and anti-dengue agents.

Keywords: *Anti-dengue drug; Docking; Schiff base ligand; 4,4'-diaminobenzanilide; p- nitrobenzaldehyde; NS3 protease- helicase.*

1. INTRODUCTION

Schiff bases are condensation products of carbonyl compound especially aldehydes or ketones with primary amines and they were first reported by Hugo Schiff [1] in 1864. Several investigations [2-7] on Schiff base showed that the presence of lone pair of electron in nitrogen atom of imine group is of considerable chemical and biological applications. Nowadays, the research field dealing with Schiff base coordination chemistry has expanded enormously. The importance of Schiff base complexes for bioinorganic chemistry, catalysis, biomedical applications, supramolecular chemistry and formation of compounds with unusual properties and structures has been well studied and reviewed [8-13]. Research on interactions between the transition metal complexes (usually Co(II), Ni(II) and Cu(II)) and DNA helps to give a knowledge about development of novel chemotherapeutics and medicine [14-17]. The interactions between small molecules with DNA can often cause DNA damage in cancer cells [18-20]. Dengue virus basically belongs to Flavivirus genus. This virus is a member of Flaviviridae family [21, 22]. The major source of the dengue virus development is human and monkey. Usually dengue affected humans have severe flu, fever, body pain like symptoms and body temperature almost reaches 40 °C which makes human to be critical. Severe headache, facial flushing and skin rash are extreme symptoms of Dengue fever and this severe condition is said to be Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). It is not possible to do the all experiment in low cost. Docking is the only way to give the corresponding exact results of newly synthesized drugs towards the targeting molecule [23-29]. Auto dock vina and discovery studio is a useful tool to study the interaction between the ligand and the target molecule. This software helps to determine the binding mode, binding affinity and also the exact stable configuration of the ligand which binds with the receptor [30-33]. Using this docking study, we can understand the coordination mode and the binding nature. It will help to design a good drug for the disease [34-37]. In this present work we plan to synthesize novel Schiff base ligand (L) and its cobalt(II), nickel(II) and copper(II) metal complexes, which were treated with human DNA topoisomerase I (PDB: 1SC7) and Dengue NS3 protease-helicase bi-functional enzyme (PDB ID: 2VBC) by docking. The biological applications like cytotoxic screening, DNA binding ability using electronic spectral studies and anti-tumor activity using MTT assay.

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2. MATERIALS & METHODS

All the chemicals used in this work were purchased from commercial sources and used the chemicals without further purification. 4,4'-diaminobenzanilide, *p*-nitrobenzaldehyde, cobalt chloride hexahydrate, nickel chloride hexahydrate and copper chloride dihydrate were bought from Sigma Aldrich, USA and used as received. Solvents used in the present research were purchased from Merck and used without further purification.

^{1}H-NMR spectrum of the synthesized Schiff base ligands have been recorded in DMSO (d_6) by using TMS as an internal standard on a Bruker Advance DRX 300 FT-NMR instrument. The EI-Mass spectra were recorded using JEOL DX-303 EI mass spectrometer at Indian Institute of Technology, Chennai. Infrared spectra of the solid samples were recorded in JASCO/FT-IR410 spectrometer in the range of 4000 – 400 cm^{-1} at 16 scans/min. Potassium bromide disc method was employed for sample preparation. Electronic spectra of the complexes were recorded using Perkin Elmer Lambda-25 UV-Vis. spectrophotometer using DMSO as solvent in the range of 200-800 nm. The room temperature X- band EPR spectra of the copper complex in DMSO were recorded on Varian E-4 X-band spectrometer using DPPH as the g-marker at Indian Institute of Technology, Chennai.

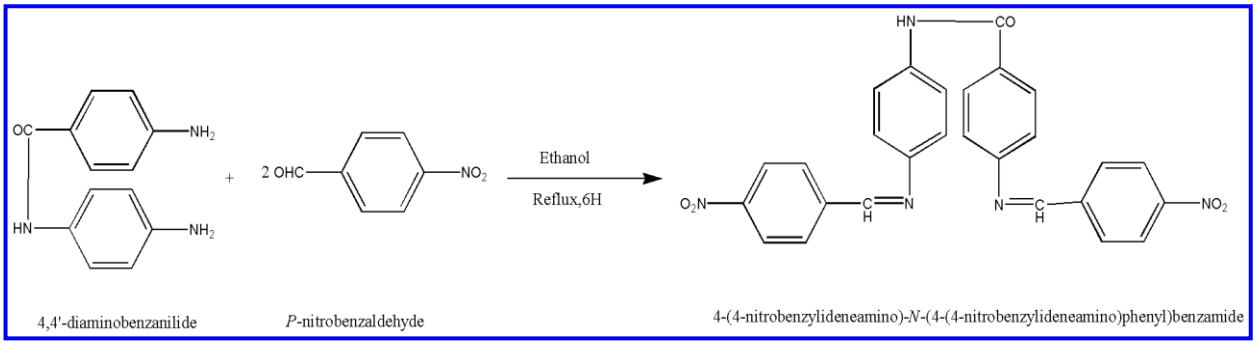
EXPERIMENTAL PROCEDURES

Preparation of Schiff base ligand L

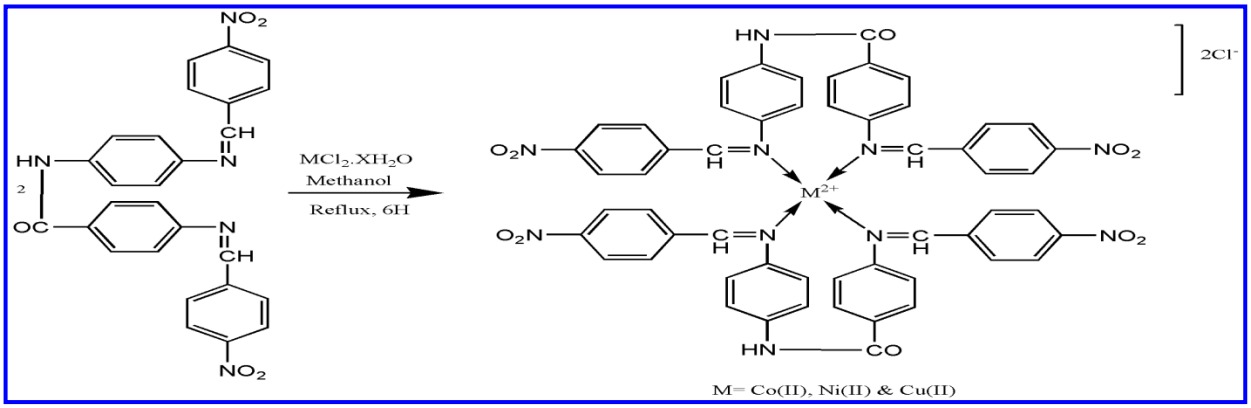
A hot solution of 1.136 g (5 mmol) 4,4'-diaminobenzanilide in 20 mL methanol was added slowly to a hot stirring solution of 1.5112 g (10 mmol) *p*-nitrobenzaldehyde in 20 mL methanol. The above mixture was stirred under reflux for 5 hours. On cooling to room temperature, the Schiff bases obtained are filtered, washed with diethyl ether and dried *in vacuo*.

Preparation of Cobalt(II), Nickel(II) and Copper (II) Schiff base (L1) metal complexes

To the hot stirring solution of the 0.9869 g (2 mmol) Schiff base ligand L2 in 20 mL of methanol the corresponding metal(II) chlorides [$CoCl_2.6H_2O$ (0.237 g, 1 mmol), $NiCl_2.6H_2O$ (0.2379 g, 1 mmol) and $CuCl_2.2H_2O$ (0.170 g, 1 mmol)] in 20 mL of methanol were added, stirred under reflux for 6 hours. Then the product obtained was filtered, washed and dried *in vacuo*.



Scheme 1. Preparation of Schiff base Ligand L



Scheme 2. Preparation of Schiff base metal complexes

DNA Binding Studies using electronic absorption spectra

Electronic absorption spectrum of the complex was recorded before and after addition of CT-DNA in the presence of 50 mM Tris-HCl buffer (pH 7.5), Tris-hydrochloride (197 mg, 5mM) and sodium chloride (730 mg, 50 mM) were accurately weighed and made up to 250 mL in a standard measuring flask using double distilled water. The pH of the solution was adjusted to 7.5 using 1 mM sodium hydroxide solution with the help of pH meter before making up to the mark. A fixed concentration of metal complexes (10 μM) was titrated with incremental amounts of CT-DNA over the range (0 – 200 μM).

Cytotoxicity screening analysis

The stock culture of bacteria was revived by inoculating in broth medium and grown at 37° C for 18 hrs. The Lysogeny broth (LB) Agar plates were prepared and wells were made in the solidified LB agar plate. Each plate was inoculated with 18 h old cultures (100 μL, 10^{-4} CFU) and spread evenly on the plate. After 20 min, the wells were filled with compound at different concentrations. Standard compound plate was also prepared in the same manner. All the plates were incubated at 37° C for 24 h and the diameter of inhibition zone were noted.

MTT assay

To determine the cytotoxic effect of Schiff base copper complexes, cell viability study was done with the MTT reduction assay. Hep-2 cells were seeded in a 96-well plate at the density of $5x10^3$ cells/well. The cells were allowed to attach and were grown in 96-well plate for 24 h, in 200 μL of Eagle's minimal essential medium (EMEM) with 10% Fetal Bovine Serum (FBS) [38]. After that the media was removed and replaced with suspension of various concentrations of Schiff base copper complexes (10 to 100 mg/mL) (minimum 4 wells seeded with each concentration), the cells were incubated for 48 h . After the addition of (MTT), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (10 mL, 5 mg/mL), the cells were incubated at 37° C for another 4 h. The medium was then removed, and 200 μL of DMSO was added to each well. Optical density of the formazan product was read at 620 nm using multi well spectrophotometer. The results were given as mean of four independent experiments. OD value was subjected to sort out percentage of viability by using the following formula

$$\text{Percentage of cell viability} = \frac{\text{OD value of the treated sample}}{\text{OD value of the control sample}} \times 100$$

Molecular Docking Study

Human-DNA-Topo-I complex (PDBID: 1SC7)] and Dengue NS3 protease-helicase bi-functional enzyme (PDB ID: 2VBC) were obtained from Protein Data Bank. Metal complexes were converted into PDB format using Mercury software [39]. The 'receptor' (DNA/Dengue) and 'ligand' (metal complexes) files were docked using Auto Dock Tools. The heteroatoms including water molecules and excess ligand were removed and also polar hydrogen atoms and Kollman charges were added to the receptor molecule to investigate the binding nature. In the binding was enclosed in a box which having number of grid points in (x) x (y) x (z) directions of 40 x 40 x 40 and a grid spacing of 0.35 Å. Docking studies were conducted using Auto Dock Tools (ADT) version 1.5.4 and finalized by Auto Dock vina programme. The docked structures were exposed using Discovery studio software.

3. RESULTS & DISCUSSION

^{1}H-NMR Spectral Studies and Electron Ionization Mass Spectral analysis

The ^{1}H NMR spectra of the ligand L is provided in the Fig. 1. The appearance of signal at 8.477 ppm is attributed to azomethine group [40] and the signal for amido proton appeared at 8.513 ppm [41]. The atomic protons of the ligand appeared in the range 7.2 – 7.9 ppm, which confirms the formation of the ligand. The EI mass spectra of ligand L is given in Fig. 2 which has the molecular ion M^+ peak at 492.9514 corresponding to the molecular weight of the ligand L. The peaks at 474.9596, 457.0669, 373.0051, 353.0565, 248.9471, 141.9279, 114.0282, 103.04 and 76.1198 corresponds to various fragments of ligand L.

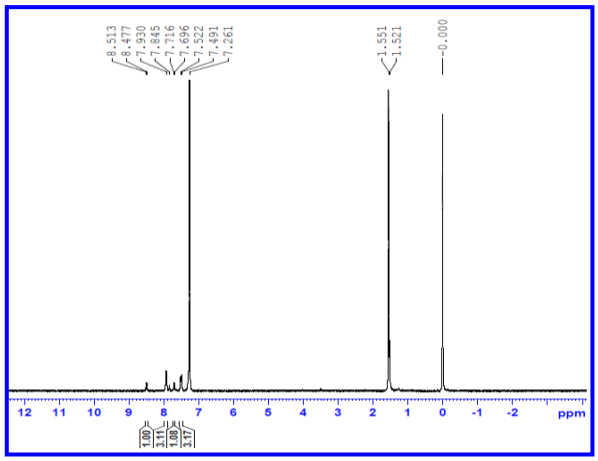


Fig. 1: ^{1}H NMR spectrum of L

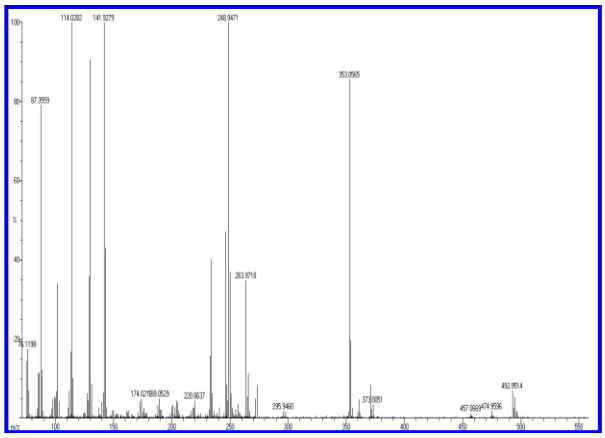


Fig. 2: EI-Mass spectrum of L

FT-IR Spectral Studies

The FT-IR spectrum of the Schiff base ligand L is given in the Fig. 3 which has a strong band at 1598 cm^{-1}, which is attributed to the imino stretching frequency of the Schiff base ligand L [42].

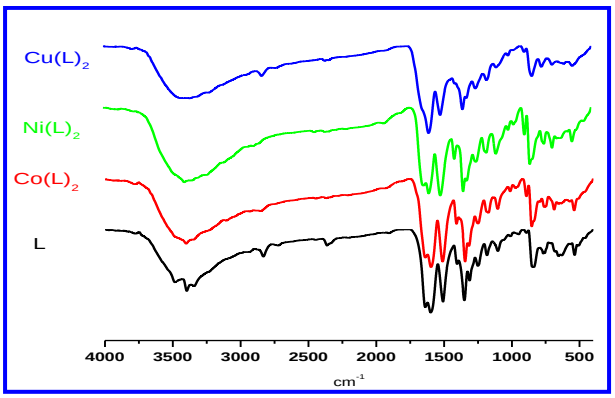


Fig. 3: FT-IR Spectra of Schiff base ligand L1 and its complexes

There is a strong peak at 3396 cm^{-1}, which is the characteristic N-H stretching frequency of the secondary amide group of *4,4'*-diaminobenzanilide moiety. The band around 3000 cm^{-1} is assigned to C-H stretching of aromatic group. The peak at 1638 cm^{-1} is due to the stretching of C=O group of 4,4'diaminobenzanilide moiety. The band around 1500 cm^{-1} and 1349 cm^{-1} are attributed to the symmetric and asymmetric stretching frequencies of N=O group [43]. The band at 838 cm^{-1} is due to the para substituted aromatic deformation frequency. This confirms the effective condensation of 4,4'-diaminobenzanilide and *p*-nitrobezaldehyde. In order to study the binding mode of the Schiff base ligand L2 to the metal in the complexes, the IR spectrum of the free ligand is compared with those of the complexes. The band near 1598 cm^{-1} is shifted to lower frequencies in complexes. This clearly indicates the coordination of the imino-nitrogen to the metal center [44]. Further the IR spectra of the complexes show some new sharp signals in the region 462 cm^{-1}, 464 cm^{-1} and 463 cm^{-1} for $Co(L)_2$, $Ni(L)_2$ and $Cu(L)_2$ complexes respectively, which corresponds to metal-nitrogen stretching formed by the coordination of imino nitrogen and metal centers [45]. Thus the FT-IR spectra confirm the formation of Schiff base metal complexes.

Electronic spectral analysis

The electronic spectra of complexes $Co(L)_2$, $Ni(L)_2$ and $Cu(L)_2$ are recorded in the range of 200 nm-800 nm in DMSO and are provided in Fig. 4.

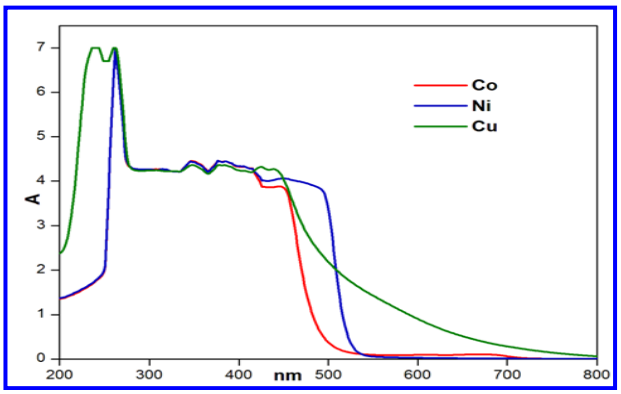


Fig. 4: UV-Vis Spectra of Schiff base metal complexes

The spectral profiles below 350 nm are similar for all the complexes and are ligand centered transitions (π-π^* and n- π^*) of benzene and non-bonding electrons present on the nitrogen of azomethine group in the Schiff base complexes [46]. In the electronic spectrum of the cobalt complex, the band around 380 nm is assigned to LMCT [47] transition while the d-d band observed in the lower energy region around 420 nm is assigned to the combination of $^2B_{1g} \rightarrow {}^1A_{1g}$ and $^1B_{1g} \rightarrow {}^2E_g$ transitions. This transition represents a square planar geometry [48-49]. In the electronic spectrum of the nickel complex, the band around 360 nm is assigned to LMCT transition. Additionally a broad band observed in the lower energy region around 370 – 410 nm is assigned to d-d $^1A_{1g} \rightarrow {}^1B_{1g}$ transition. This transition represents the square planar geometry for the nickel complex [50]. The electronic spectrum of copper complex shows three major peaks at around 271, 325 and 450 nm. The first two peaks can be attributed to the transitions from ligand moiety. The band at ~325 mm is due to the LMCT transition. In addition, the charge transfer transitions from metal to ligand or ligand to metal may also be the reason for the emergence of this band in the electronic spectrum of copper complex. Besides, the expected d-d transition band is noted at ~450 nm which is attributed to the combination of $^2B_{1g}$

→ ^2E_g and $^2B_{1g}$ → $^2B_{2g}$ transitions. This further stands as the evidence for the square planar geometry of d^9 Cu(II) system [51].

EPR spectral analysis

The EPR spectra of copper complex provide information of importance in studying the metal ion environment. The copper complex $Cu(L)_2$ exhibits an isotropic signal, without any hyperfine splitting, with g_{iso} = 2.104 as shown in the Fig. 5. The g value obtained in the present study when compared to the g value of a free electron, 2.0023, indicate an increase of the covalent nature of the bonding between the metal ion and the ligand molecule [52]. Isotopic lines usually results due to occupancy of the unpaired electron in a degenerate orbital in square planar geometry.

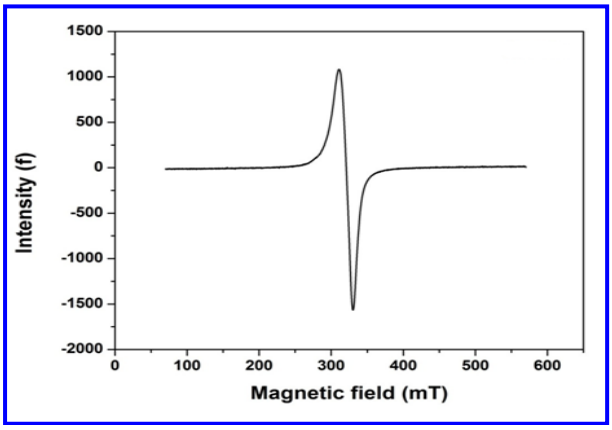


Fig. 5: EPR Spectrum of $Cu(L)_2$

Cytoxicity screening

There are several methods for assessing the carcinogenic or mutagenic properties of the given chemical structure. The method followed here is a bacteria strain based assay, which includes E. coli AB1157 which is a wild-type strain proficient in DNA damage repair. The bacterial strain is incubated with the compounds of interest for the analysis of any associated lethl effects. On incubation with the compounds, any free radical generation by the compounds lead to the lethality of the cells [53]. This cytotoxic potentiality of the compound will be displayed in terms of zone of inhibition. The cytotoxic screening analysis of the complexes showed that copper complex alone exhibit exellent cytotoxicity screening effects at MIC value of 0.25 mg.

Table 1. Zone of inhibition (mm) for Cytotoxicity Screening analysis

Compounds	0.125 mg	0.25 mg	0.5 mg	1.0 mg	2.0 mg	MIC (mg)
$Co(L)_2$	0	0	3	7	10	0.5
$Ni(L)_2$	0	0	0	0	5	2.0
$Cu(L)_2$	0	2	4	5	10	0.25
Stannous chloride	0	0	3	8	15	0.5

DNA binding studies using electronic spectra

The interactions of metal complexes with DNA are of interest in order for the development of chemotherapeutic agents. Electronic aborption spectroscopy is one of the most useful techniques for DNA binding studies of metal complexes. The interactions of copper complex $Cu(L)_2$ with CT-DNA were investigated by UV-Vis absorption titrations and is represented in Fig. 6.

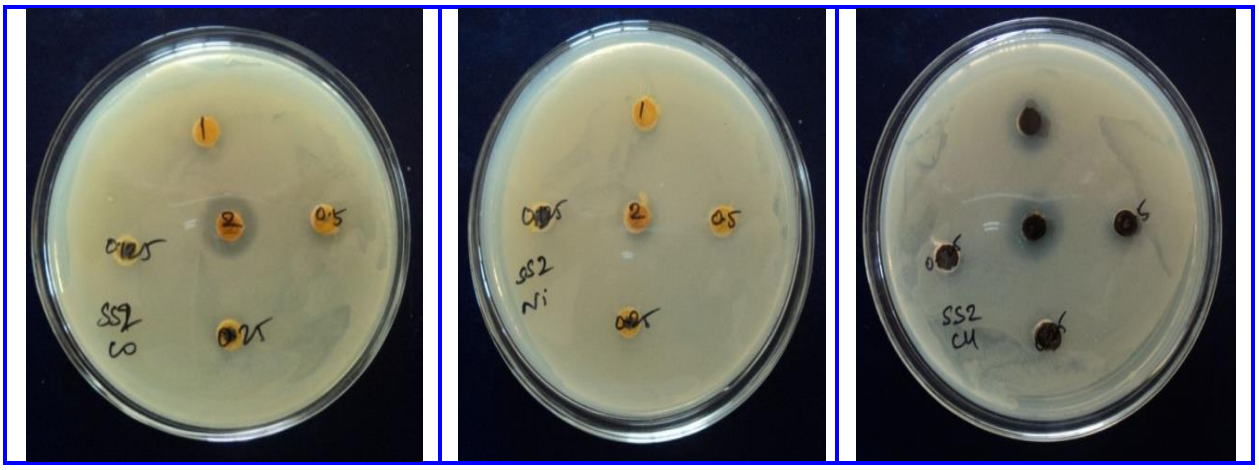


Fig. 6: Cytotoxicity Screening analysis of complexes

Upon addition of increasing amount of CT-DNA from 0 – 200 µL, a significant "hyperchromic" effect of the intra ligand bands at 257.8 – 300 nm was accompanied by a red shift of 2 – 7 nm, indicative of the breakage of the DNA helix [54,55]. There is no appreciable change in the charge transfer band. As the concentration of the DNA was increased, the absorption bands of the copper complex initially showed hyperchromism, but on further increasing concentration, hyperchromism with blue shift is obtained (fig. 7).

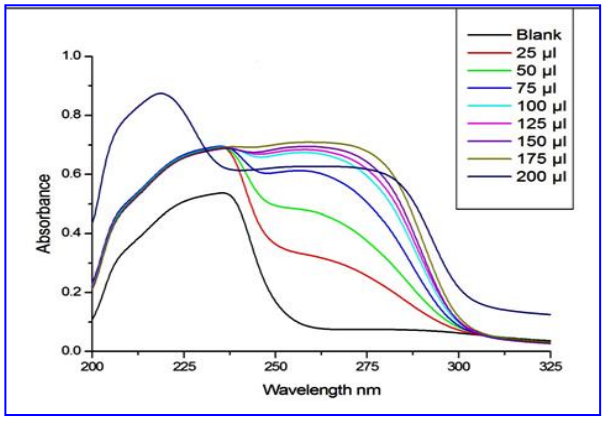


Fig. 7: DNA Binding interaction of $Cu(L)_2$

The invitro cytotoxicity of the copper complex $Cu(L)_2$ was evaluated against Larynx Cancer Cells Hep_2 and given in fig. 8. The IC_{50} (the concentrations that inhibited in 50% the cellular proliferation) of the studied complex is 40µg.

Molecular docking with human DNA topoisomerase I

Human DNA topoisomerase I and II were the selective targeting area for synthesizing the anticancer drug [56]. The molecular docking of Cobalt(II), Nickel(II) and Copper(II) complexes were performed to determine the value of binding affinity and the selected binding residue which along with the sterically suitable conformations. The low value of the binding energy shows the more effective binding affinity between the 'receptor' and the 'ligand' molecules. The various conformations of docked molecular complexes were analyzed in terms of binding energy, hydrogen bonding and hydrophobic interaction between receptors and the acceptor. More negative value of the relative binding values suggests that the interaction between the DNA and ligand is so strong, due to the extended aromatic ring. Phenyl ring has higher free binding energy which gives a better binding affinity value comparing with a compound containing bipyridyl ring. From these work mononuclear complexes gives better results towards the HDNA. The binding energy values of the Co(II), Ni(II) and Cu(II) complexes respectively are -11.6, -6.9 and -10.4 kcal mol^{-1} towards human DNA topoisomerase I. This shows that Cobalt(II) and Copper(II) molecule easily binds with the DNA helix and the Nickel(II) complex preferred to bind with the outermost protein's amino acid residue. The docked images were shown in Fig. 9.

NS3 protease-helicase (dengue virus) is a very important target area which should be docked. Cobalt(II), Nickel(II) and Copper(II) complexes exhibit very low binding energy value, it means that complexes having very high binding affinity towards the NS3 protease-helicase. The distance between the selected receptor to targeted molecule is also low. The binding energy values of the Co(II), Ni(II) and Cu(II) complexes are-12.7, -12.9 and -12.6 kcal mol^{-1}respectively. The binding interactions were shown in fig.10. From the theoretical point of view these complexes are consider to be a good anti-dengue drug.

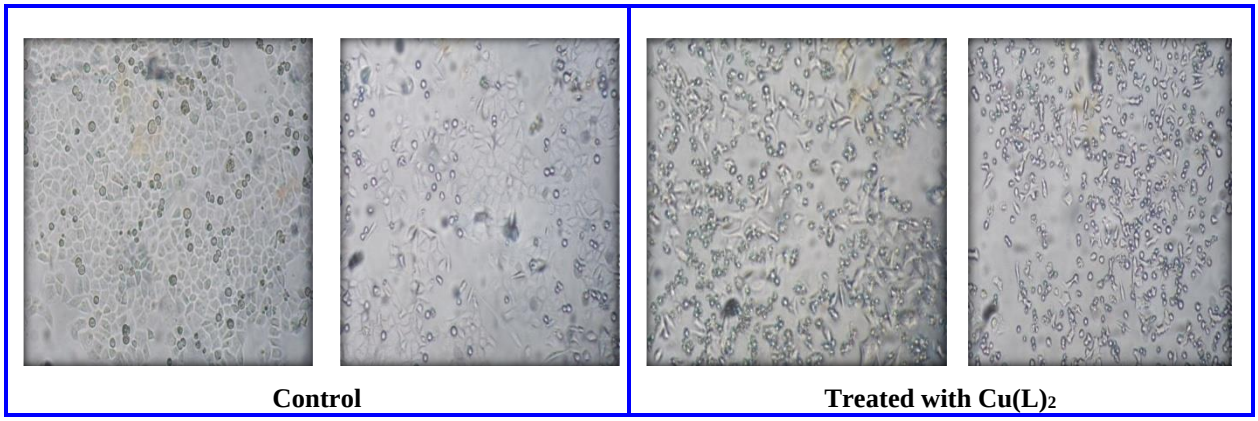


Fig. 8: Anti-Tumor activity of $Cu(L)_2$

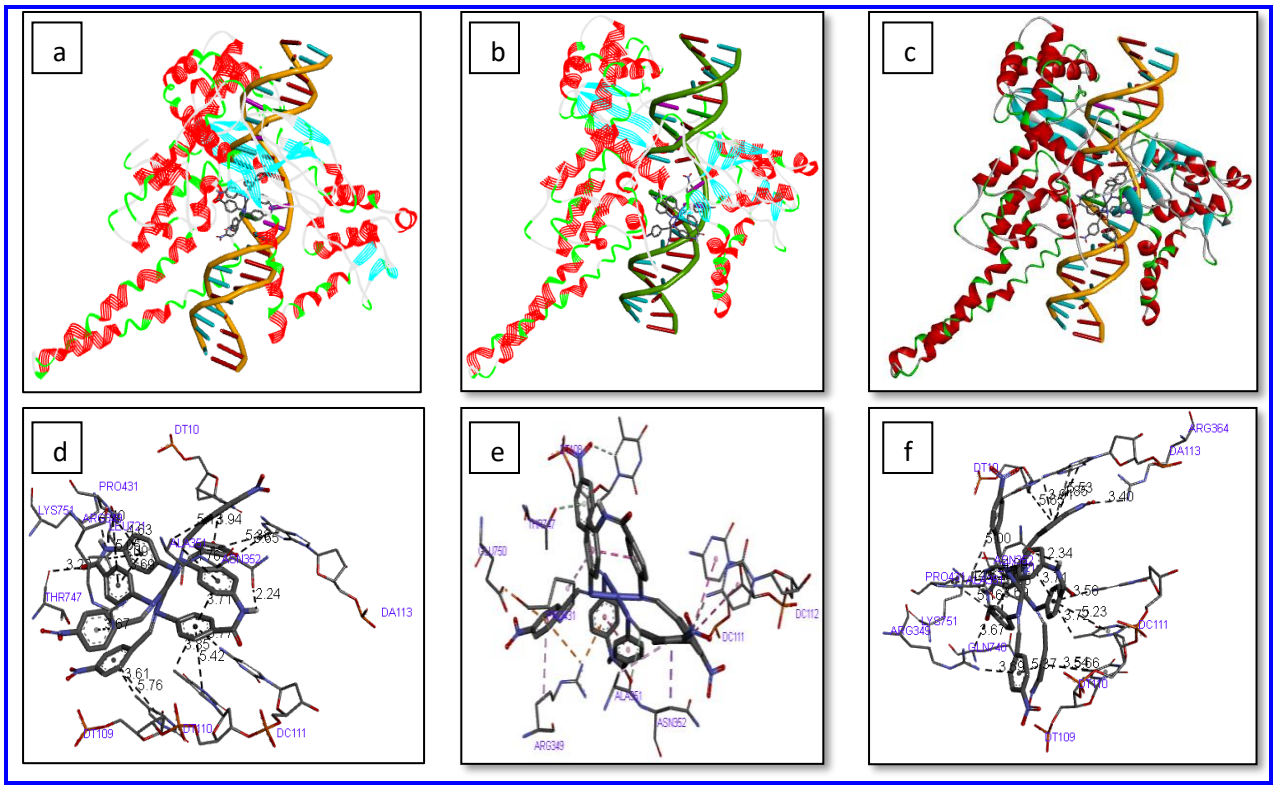


Fig. 9: (a), (b) and (c) demonstrate the Co(II), Ni(II) and Cu(II) complexes bind with active site of human DNA topoisomerase I, (d), (e) and (f) demonstrate the Co(II), Ni(II) and Cu(II) complexes bind with selective Nucleotide of human DNA topoisomerase I

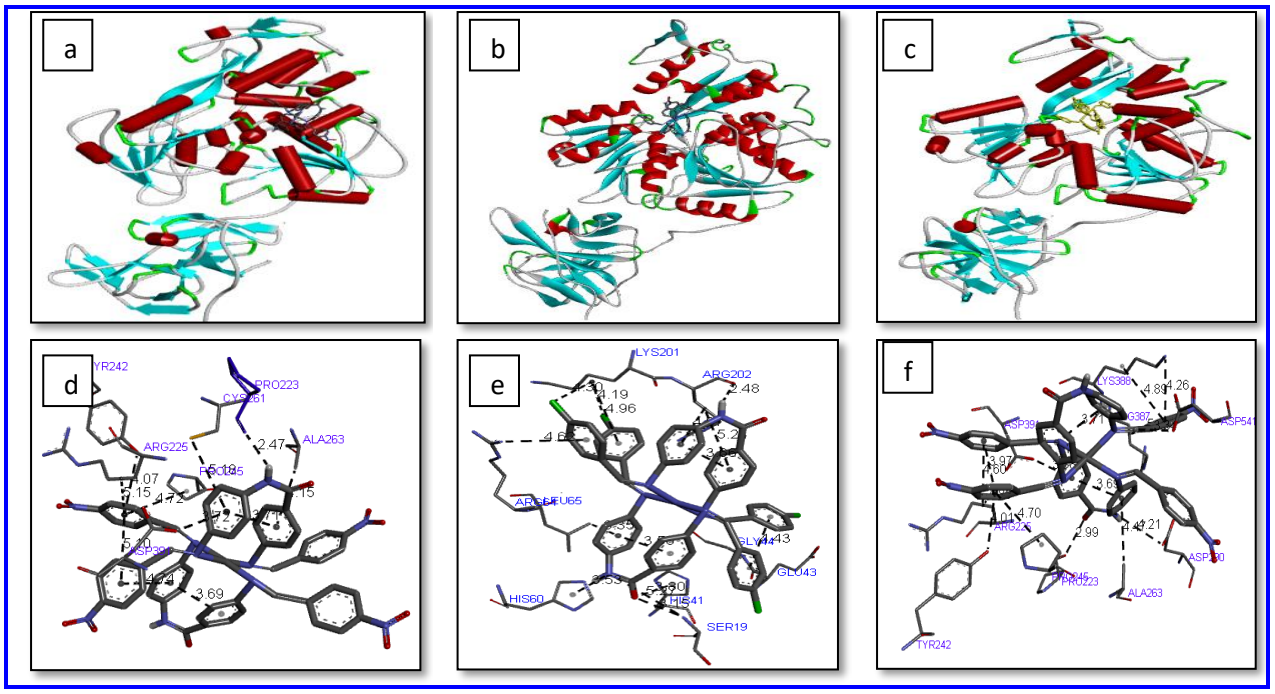


Fig. 10: (a), (b) and (c) demonstrate the Co(II), Ni(II) and Cu(II) complexes complexes bind with active site of NS3 protease-helicase, (d), (e) and (f) demonstrate the Co(II), Ni(II) and Cu(II) complexes bind with selective Amino acid residue of NS3 protease-helicase

4. CONCLUSION

A new Schiff base ligand L which contains several phenyl rings and its cobalt(II), nickel(II) and copper(II) complexes were synthesized. The formation of the ligand and its metal complexes were confirmed by various spectroscopic techniques. The biological applications of the synthesized complexes were carried out by Cytotoxic screening analysis, DNA binding Ability by using electronic spectra and Anti-Tumor activity by MTT assay. The results obtained showed that among the synthesized complexes copper complex has potential biological activity. The docking studies were carried out using synthesized metal complexes with human DNA topoisomerase I (PDB: 1SC7) and Dengue NS3 protease-helicase bi-functional enzyme (PDB ID: 2VBC) using Auto Dock vina and Discovery studio software. The binding energy values of the Co(II), Ni(II) and Cu(II) complexes showed that these types of compounds can be act as an potential Anti-Dengue and Anticancer agent

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